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THE UNIVERSITY OF ALBERTA

COMPARISON OF INTRAPORTAL AND INTRASPLENIC CANINE PANCREATIC FRAGMENT AUTOTRANSPLANTATION

by

© WILLIAM RAND FORGIE

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF MASTER OF SCIENCE
IN
EXPERIMENTAL SURGERY

DEPARTMENT OF SURGERY

EDMONTON, ALBERTA

FALL 1986

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled COMPARISON OF INTRAPORTAL AND INTRASPLENIC CANINE PANCREATIC FRAGMENT AUTOTRANSPLANTATION submitted by WILLIAM RAND FORGIE in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE IN EXPERIMENTAL SURGERY.

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ABSTRACT

Islet transplantation in the rat is most successful when islets are infused intraportally to the liver. In the dog, the commonest large mammal model, the optimum site for transplanting pancreatic fragments is not known. Transplantation of pancreatic fragments to the liver in man has been complicated by portal hypertension (PH) and disseminated intravascular coagulation (DIC).

Twenty six dogs underwent total pancreatectomy with 6 dogs serving as apancreatic controls. The remaining dogs received an autotransplant of pancreatic fragments after gland preparation. The pancreas was digested with collagenase by retrograde ductal perfusion; then chopped, dispersed, filtered and washed prior to transplantation. Graft was embolized to the spleen by retrograde hilar vein infusion over 10 min. (n=6), or via intraportal infusion to the liver over 20 min. (n=6). Slow, intermittent infusion over 60 min. or systemic anticoagulation with 100-150u heparin/kg was used individually in two dogs and then the two techniques combined (n=6).

Apancreatic controls died at 6.3 $\pm$ 1 days postoperatively. Five of 6 intrasplenic recipients were long term survivors with K values (% decline in glucose/minute) at 1, 3 and 6 months of 1.5, 1.4 and 1.4 respectively (preop K value = 2.8). Weight loss at 6 months was 1%. PH or DIC was not evident. Five of the intraportal infusion control dogs died within 24 hours of transplantation. PH with pressures 49 cm H_2O was seen but there were no clinical or histological signs of DIC occurring. PT, PTT and fibrinogen levels did not change significantly. In one dog FDP's were present (10-40 ug/ml). The

platelet count immediately postinfusion was significantly decreased (203,000 to 140,000, $p < 0.05$) but 30 min. later the difference was no longer significant (203,000 to 161,000, $p > 0.10$). Individual dogs did not survive slow, intermittent infusion or systemic anticoagulation given prior to intraportal infusion but when the two techiques were combined 6 of 6 dogs survived and four were long term survivors with K values at 1, 3 and 6 months of 1.4, 1.4 and 1.5. Weight loss at 6 months was 20% - significantly greater than the intrasplenic group. Maximum portal pressure elevations were significantly less than the control group (29.6 vs 49 cm H_2O) and were only transient. DIC was not seen.

Intraportal autotransplantation of canine pancreatic fragments to ameliorate diabetes mellitus is possible when slow, intermittent infusion is combined with systemic anticoagulation. However, the liver has not been shown to be a superior transplantation site for pancreatic fragments compared to the spleen.

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INTRODUCTION

Diabetes Mellitus

Diabetes mellitus (DM) is a disease affecting pancreatic islets of Langerhans resulting in a relative or absolute deficiency in the formation and release of insulin. It has profound systemic and metabolic effects as a result of disordered carbohydrate metabolism. Diabetes affects 2-3% of the population and evidence suggests its incidence is increasing.[1]

Approximately 10,000 new insulin-dependent diabetics are diagnosed each year (U.S.A.) and about half of those can expect long-term complications, affecting both the quality and quantity of life. Diabetes is the leading cause of blindness in young adults and diabetics are 25 times more prone to fatal kidney disease, 5 times more prone to limb gangrene (that often leads to amputation) and pregnant diabetics are at 20 times a greater risk of death than their non-diabetic counterparts. Perinatal infant mortality approacnes 20%. The average life expectancy is approximately 1/3 less than that of the general population. The above complications, secondary to altered carbohydrate metabolism, are a result of pathological processes affecting both large and small blood vessels within all organs including the peripheral vascular system in general and the nervous system.[2]

The discovery of insulin by Banting and Best[3] in 1922 dramatically changed the outlook on a disease that was otherwise fatal within 2-3 years of diagnosis.[4] However, experience has now shown that the standard therapy of once or twice daily self-administered

insulin does not prevent complications in the long-term surviving diabetic. Further research attempts to more clearly understand insulin deficiency and discover alternate therapeutic techniques of insulin administration.

Present treatment alternatives are aimed at acheiving better metabolic control with administered insulin. Complications in the surviving diabetic are thought to be mainly a result of disordered metabolism secondary to insulin deficiency; as opposed to a more generalized defect, either inherent or acquired, affecting not only insulin secretion but the microvasculature as well. Evidence from both animal models[5,6,7] and clinical trials or experience[2] favours the former theory.

If insulin deficiency is the underlying problem, then its administration in a manner to cause relative normoglycemia should retard or even reverse the complications of D.M. But once or twice daily self-administration of insulin allows fairly wide fluctuations in glucose levels not normally seen in the otherwise healthy person. Proof that "tight control" (relative normoglycemia) of diabetics alters the long-term prognosis is not conclusively available but many animal experiments and extended clinical trials suggest that it is the goal to aim for.[2,4,8]

Alternatives to Present Insulin Therapy

The current approaches to maintaining normoglycemia and avoiding the wide fluctuations in blood glucose include:

(i) frequent, multiple injections of short acting insulin

(ii) use of continuous infusion pumps containing injectable insulin

(iii) transplantation of islet tissue as either whole organ or tissue suspension.

The method of frequent, multiple injections depends on repeated glucose sampling which is inconvenient but in the well motivated patient it is a safe and immediately available technique.[9] Continuous infusion pumps, again for the selected patient, hold promise as a therapeutic alternative and various prototypes are available in selected clinics.[9] However, the lack of feedback control carries the risk of inadvertent hypoglycemia. This has been a major and potentially fatal drawback.[9]

Transplantation of islet tissue is gaining interest and each year proves more and more feasible. The advantages of transplanting autoregulatory insulin-secreting tissue are obvious and although it may not provide an absolute cure, its objective is to avoid the complications that presently occur in spite of exogenous insulin.[2,10]

Animal Models for Islet Transplantation

Several animal models have been developed for islet transplantation but satisfactory results in some of these has led to only limited success when applied clinically.[11,12]

In various animal models including rodents,[5,7,13,14,15] primates,[16,17,18], dog[19,20,21] or pig[22] it has been shown that transplanted islet tissue can not only normalize glucose homeostasis

but even reverse diabetic lesions and complications.[5,14]

Most animal trials involve transplantation of pure islets or dispersed pancreatic fragments containing islets. But in humans, segmental gland transplanation has been most successful.[23,24] However, solid organ transplantation is hampered by technically difficult vascular access and disposing of exocrine secretions. In addition, segments of pancreas have very limited storage capabilities.

Transplantation of pure islet tissue or islet-bearing pancreatic fragments is attractive because of:

(i) their relative ease in handling

(ii) the potential ability to modify immune recognition and therefore decrease required immunosuppression

(iii) the near or total elimination of exocrine tissue and

(iv) the ability to store tissue by cryopreservation.[25]

The major disadvantage is the inability to obtain adequate numbers of viable islets.[26]

Although recorded efforts in pancreas transplantation began as early as 1893, not until the 1920's was it realized the exocrine component threatened the viability of islets.[27] This led to an intensive search to separate one from the other. The first relatively pure islet tissue obtained was from fetal or neonatal whole gland preparations. It was known this tissue contained a far greater proportion of developed endocrine tissue to undifferentiated acinar elements.[28,29] But rapid advances in obtaining large numbers of islets were not seen until Moskalewski[30] in 1965 developed a

technique using collagenase to digest chopped guinea pig gland that helped to separate islets from acinar tissue. Further improvements were made by Lacy and Kostianovsky[31] and in 1972 Ballinger and Lacy reported the first significant success in ameliorating diabetes by the intraperitoneal or intramusclar isotransplantation of islets in streptozotocin-diabetic rats.[32] Reckard soon confirmed the intraperitoneal technique.[33] Kemp demonstrated that intraportal infusion of pure islets was superior to intraperitoneal transplantation;[34] this was later confirmed by Matas et al.[6] Independently Feldman[35] and Kretschmer[36] showed an improved response following intraportal infusion compared to intrasplenic transplantation in rats. As a result the preferred site of transplanting pure islet tissue from multiple isogeneic donors in small mammals is the liver.

As none of the rodent protocols for islet isolation could be successfully extended to the human pancreas[6,26] an animal of intermediate size with a pancreas more closely resembling the humans was required for experimentation. Among the monkey, pig or dog the latter has received the most attention because of availability and relative ease in handling.

Mirkovitch and Campiche discovered that when working with the dog pancreas complete separation of islet and exocrine tissue was not necessary.[37] Incomplete separation allowed recovery of enough islet tissue from a single gland to reverse diabetes when autotransplanted into the spleen of a pancreatectomized dog.[38,39] With individual modifications to their technique the method of incomplete separation

of endocrine and exocrine tissue has been confirmed[40,41] and used by others.[42-45] This technique begins with gland distention by either retrograde ductal infusion or direct tissue injection. Following this the gland is minced and then digested in collagenase. After rinsing, the tissue is ready for transplantation.

An alternate procedure was developed by Horoguchi and Merrel in 1981.[46] They employed ductal distention of the gland but included collagenase in the ductal perfusate to aid digestion, followed by mincing and fragment filtrations and a further trypsin digestion. They claimed a 50% recovery of islet tissue following total pancreatectomy and a 6 fold purification of endocrine to exocrine tissue. Tissue was autotransplanted to the liver by portal vein injection or to the spleen and this reversed the diabetic state in 5 of 7 dogs. However, neither the degree of normoglycemia or length of survival were mentioned.

One of the more successful models for transplanting islet-bearing pancreatic fragments was developed by Warnock et al.[19] in 1983. With several modifications to Horoguchi et al.'s technique, all isotransplants to the spleen were immediately normoglycemic and 7 of 9 dogs remained so until sacrifice at 5 months. His method consistently conserved a sufficient amount of viable endocrine tissue to immediately reverse hyperglycemia and assessment by glucose challenge showed good and persistent graft function. Only collagenase digestion was employed and gland mincing was more uniform using counter-rotating chopping blades.[6] Tissue was infused into the spleen by retrograde injection in cannulated hilar veins.

In the autotransplantation model of any large mammal the question of optimum transplantation site for islet tissue has not been thoroughly addressed.[11] Certainly in the rodent embolization to the liver via the portal vein has proven to be the best but extension of this to the dog[36,43,47] (or even the human in some early clinical trials[24,48,49,50]) has produced complications and raised questions about its potential superiority. The advantages of intraportal transplantation - availability and ease; secretion of insulin and other islet hormones with the portal-digestive axis; and the rapid attainment of a rich vascular supply - remain to be proven.

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AUTOTRANSPLANTATION OF PANCREATIC FRAGMENTS IN THE DOG: A COMPARISON OF INTRAPORTAL AND INTRASPLENIC SITES

Introduction

Clinical transplantation of pancreatic islet tissue has been proposed as a method to prevent the long-term complications of insulin-dependent diabetes mellitus (DM). This transplantation approach still does not have a satisfactory large mammal model to follow.[1]

Ballinger and Lacy[2] developed a successful islet isolation and transplantation technique in the rat. The importance of maintaining insulin secretion within the portal-digestive axis was shown by Brown[3] and the liver was identified as the superior transplantation site.[4,5] Similar research protocols were developed in the dog.[6-11]

The gland preparation of Mirkovitch and Campiche (gland distention, mincing and collagenase digestion) results in pancreatic fragments containing islets but contaminated with exocrine tissue. When autotransplanted to the spleen of the donor dog hyperglycemia is satisfactorily reversed. Intraportal infusion[7,12] has given similar results. While Kolb[8] found no differences between hepatic and splenic transplantation sites, Kretschmer[9] found the latter to be superior. Lorenz[10] transplanted isolated pure islets intraportally but his partial pancreatectomy/ streptozotocin-diabetic dogs make his results difficult to evaluate and compare.

A complication of intraportal infusion using the larger pancreatic fragments (compared to smaller, pure islets) is portal hypertension (PH).[7,12] Heparin has been used to prevent the

clotting that accompanied PH and the resulting venous stasis.[8,9,11] Increases in portal pressure occurred but usually were transient. Disseminated intravascular coagulation (DIC)[11] and systemic hypotension[13] have also been reported as complications.

The infusion of pancreatic fragments intraportally in humans has given unsatisfactory control of hyperglycemia and similar complications - PH, DIC and systemic hypotension.[11,13-19]

An alternate method of pancreatic fragment preparation is ductal distension and perfusion/digestion with collagenase. When autotransplanted to the spleen in the pancreatectomized dog this has resulted in immediate control of hyperglycemia and superior long-term follow up.[20] Attempts to infuse the pancreatic fragments intraportally led to severe, sustained portal hypertension and the dogs early death.[21] The purpose of this study was to develop a method of intraportal autotransplantation using the Warnock et al.[20] pancreatic fragment preparation and to study the complications and long-term results compared to intrasplenic autotransplantation.

Materials and Methods

Outbred, adult mongrel dogs, 1-2 years of age weighing 15-25 kilograms were studied. Criteria of the Canadian Council on Animal Care were followed.

Perioperative Care

All dogs underwent a total pancreatectomy.[22] Broad spectrum antibiotics were administered pre- and postoperatively. Subcutaneous N/S supplemented oral intake until a full diet was resumed on the

third postoperative day. Cotazyme (Organon, Toronto, Ont.) was mixed in with meat and dry meal.

Pancreatic Fragment Preparation

During pancreatectomy, the major blood supply was preserved and both pancreatic ducts were individually cannulated in situ. Immediately following excision the gland was weighed and the ductal system distended with 80 ml of chilled (4°C) Hanks balanced salt solution (HBSS - Gibco, Grand Island, N.Y.).

The pancreatic fragment preparation was similar to the protocol of Warnock et al.[20] Ten minutes of retrograde ductal perfusion with chilled HBSS was followed by a 28-32 minute perfusion with 0.4% collagenase (Sigma Type V, St. Louis, Mo.) at 30°C, limiting perfusion pressures to below 300 mm Hg. A mucoid consistency of the gland was a satisfactory end point. The gland was chopped for 90 sec[12] followed by vigorous shaking for 10 minutes in a 4°C water bath while suspended in HBSS supplemented with 100,000 units/ℓ Pencillin G; 100,000 ug/ℓ streptomycin and 200,000 KIU/ℓ Trasylol (Aprotinin, Miles Pharmaceuticals, Rexdale, Ont.). Pancreatic fragments were then filtered through a 400u screen. The shaking/filtering of remaining ulfiltered tissue was repeated twice leaving 2-3 ml to be discarded. The recovered tissue was washed x3 and resuspended in the supplemented HBSS.

Transplant Protocols

Apancreatic Controls - Six dogs did not receive an autotransplant or exogenous insulin.

Intrasplenic Autotransplant Controls - Six dogs received an autotransplant to the spleen via reflux through cannulated splenic hilar veins over a 10 minute period.[20]

Intraportal Autotransplant Controls - Six dogs received an intraportal autotransplant via a 16 gauge cannula placed within the the portal vein using the pancreaticoduodenal vein for access. A 20 minute continuous infusion was used. Four dogs received "wash" solution (20 ml) or HBSS infusions (20 ml) prior to autotransplantation.

Intraportal Infusion of Enyzme Depleted Graft - Two dogs received an intraportal infusion of graft fixed in 10% formalin and then thoroughly washed and resuspended in the supplemented HBSS. A 20 minute infusion was employed.

Intraportal Autotransplantation Using Systemic Anticoagulation or Slow Infusion - One dog was systemically anticoagulated with heparin (100 units/kg) before a 20 minute infusion and another dog received a slow, intermittent infusion over 45 minutes.

Intraportal Autotransplantation Using Slow, Intermittent Infusion with Systemic Anticoagulation - Six dogs were systemically anticoagulated with heparin (100-150 units/kg) prior to a 60 minute infusion period. Five minute infusion intervals were each interrupted for 5 minutes with no infusion. Thirty units of heparin/ml of recovered tissue was added to the graft suspension.

Intraportal Autotransplantation Using Tissue Cultured Pancreatic Fragments - In 9 dogs, after processing the gland, the entire graft was tissue cultured using sterile technique. The media chosen was

RPMI 1640 (Gibco, Grand Island, N.Y.) using 100 ml of media per ml of tissue. The media was supplemented with heat inactivated fetal calf serum (10% v/v) (Gibco); penicillin, streptomycin and aprotinin were added in the same concentrations as they were to the HBSS during gland preparation. HEPES (Gibco) was added to a concentration of 25 mM/l as a pH buffer. Tissue was placed either in multiple static tissue culture flasks or was kept suspended in continuous motion by a magnetic spinbar assembly in a 2 l Fernbach flask. The cultures were placed in a 5% CO_2/room air humidified environment. Tissue was kept in culture for 24-48 hrs. (with a media change at 24 hrs.) and then pelleted and resuspended in supplemented HBSS for transplantation. Media pH and bacterial cultures were routinely checked.

The graft obtained was then infused over a 20-60 min. interval. The latter 5 dogs were anticoagulated when the value of this was realized.

Portal pressures were monitored continuously by a simple water manometer connected to the portal infusion catheter by IV tubing. The dogs midaxillary line was chosen as a zero reference point.

The details of coagulation parameters measured to evaluate the development of DIC are presented in Paper #2.

Follow Up

Dogs were weighed weekly and fasting plasma sugars were measured by the glucose oxidase method on a Beckman Glucose Analyzer. Values less than 150 mg% represent a non-diabetic animal. Intravenous glucose tolerance testing (IV GTT) was performed preoperatively; 1, 3 and 6 months postoperatively and at 3-6 month intervals thereafter.

K values (the per cent decline in plasma glucose per minute) were calculated from the 5, 10, 15 and 30 minute values.[23] Non-diabetic K values are > 1%/minute.

Dogs that were consistently hyperglycemic and/or had lost >20% body weight were sacrificed. Following death all dogs underwent autopsy. Tissue recovered was fixed in 10% formalin and underwent standard preparation for light microscopy using H&E stain. Islets were highlighted using Gomori's aldehyde-fuscin with Mallory's trichrome counterstain.

PTT's were done manually in a fibrometer using Automated APTT reagent (General Diagnostics, Morris Plains, N.Y.).

Statistical Analysis

Means are expressed with the standard error of the mean (SEM) unless otherwise indicated. Comparison of group results was done with paired or unpaired Student's t-tests. Differences are stated as significant when $p < 0.05$ unless indicated otherwise.

Results

Gland Preparation

The mean volume of tissue recovered and mean % recovery in each major comparison group is shown in Table 1-1. Differences in the volume of tissue recovered were not significant.

Apancreatic Controls - All dogs immediately became hyperglycemic (Figure 1) and died $\bar{x} = 6.3 \pm 1$ days post-op. At autopsy there was no residual pancreatic tissue.

Intrasplenic Autotransplant Controls - Five dogs became normoglycemic (Figure 1). Failure in one dog was due to poor graft disperson, clumping and graft necrosis; a recognized problem with the technique.[24] One dog died of a bowel obstruction one month following transplantation ($\bar{x}$ post-op glucose = 100 mg/dl). The four remaining dogs tended to regain weight lost in the first 2 weeks. Weight loss at 6 months was 1% (Figure 2). K values at 1, 3 and 6 months postoperatively are not diabetic but are significantly less than the preoperative values (Table 1-2). K values remained stable over the 6 month period indicating that graft function did not deteriorate.

PH was not a visible problem. Portal pressures measured in 2 dogs during infusion showed minimal elevations from baseline (1.5 and 2.0 cm H_2O).

Intraportal Autotransplant Controls - Portal pressure elevations occurred acutely in all dogs and were sustained in 5 (Figure 3). During the infusion there was a steady increase in pressure. The portal vessels and spleen became engorged with blood and petechiae appeared throughout the mesentery. The bowel was dusky and in spasm. A shock-like state developed in the dog with hyperventilation and tachycardia. Those dogs with sustained PH (n=5) died on the same operative day. Similar gross findings (seen during surgery) were present at autopsy. The portal tree contained no thrombus. By light microscopy most portal venules were plugged with pancreatic fragments (Plates 1-1 and 1-2). Acute hepatocellular necosis was present.

On 4 occasions infusion of the wash solution (collected at the completion of gland preparation) or plain HBSS caused no increase in

Table 1-1: Volume and Percent Recovery of Pancreatic Fragments for Infusion.

	n	Mean Volume ml ± SEM	Mean % Recovery ± SEM
Splenic Autotransplants	4 of 6	18 ± 1.4	39 ± 5.7
Intraportal Autotransplant - Controls	6	16.5 ± 1.1	28 ± 2
Intraportal Autotransplants - slow infusion/anticoagulation	6	16 + 1.0	28 ± 2
Total Gland Preparations*	32	18 ± 2.9**	32 ± 8.7**

* Including apancreatic controls; enzyme depleted grafts; slow infusion or anticoagulated dogs and tissue culture autotransplants.

** S.D.

Table 1-2: Pre- and Postoperative K Values - Intrasplenic Autotransplant Controls

	Preoperatively	Postoperatively		
	n=12*	1 month n=5	3 months n=4	6 months n=4
K (% decline glu/min.)	2.8 ± .16	1.5 ± .26	1.4 ± .35	1.4 ± .27

* Control (n=6) and intrasplenic autotransplantation (n=6) dogs.

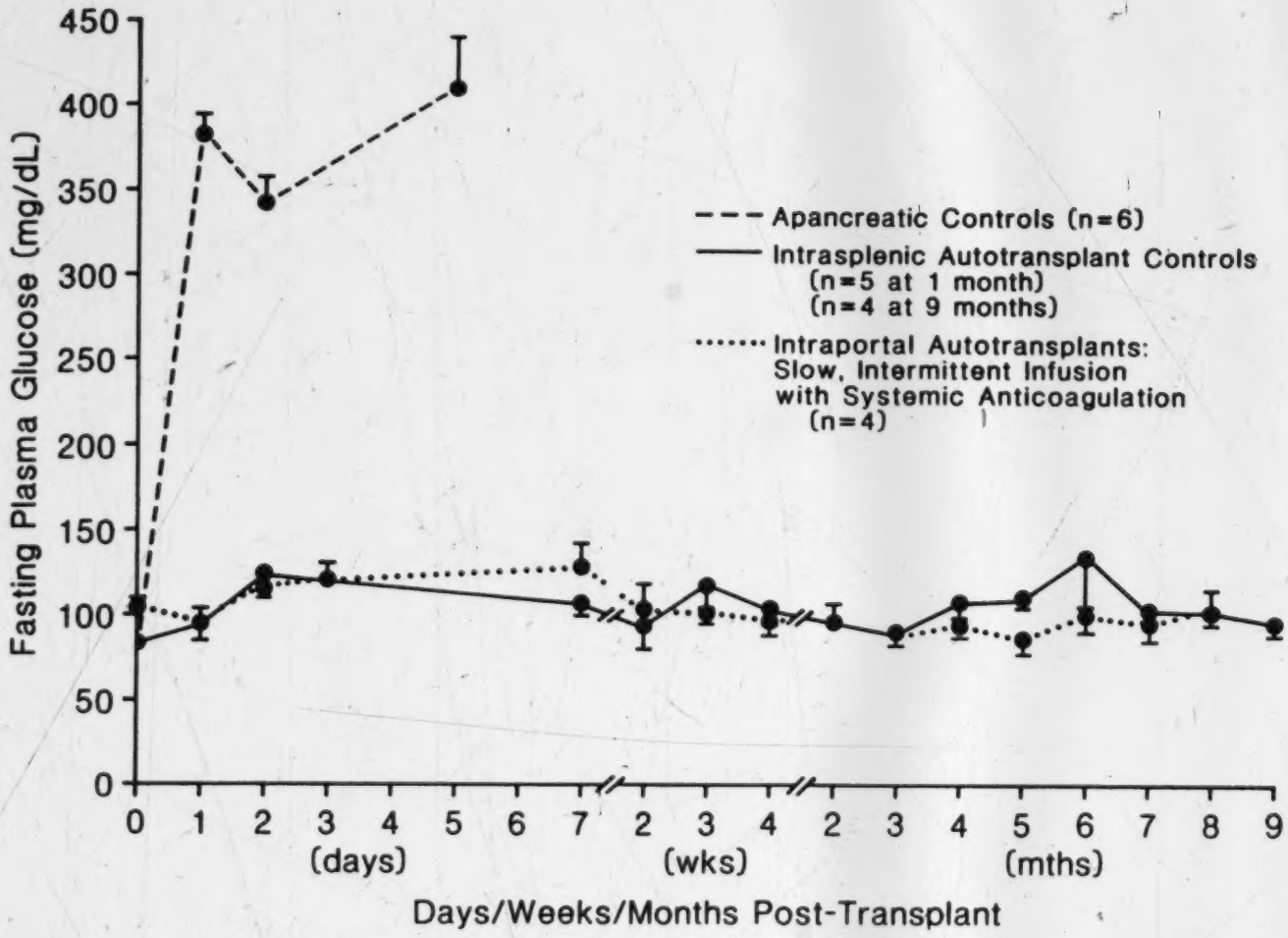

Figure 1 Comparison of postoperative fasting plasma glucose in apancreatic controls, intrasplenic autotransplant controls and successful intraportal autotransplants.

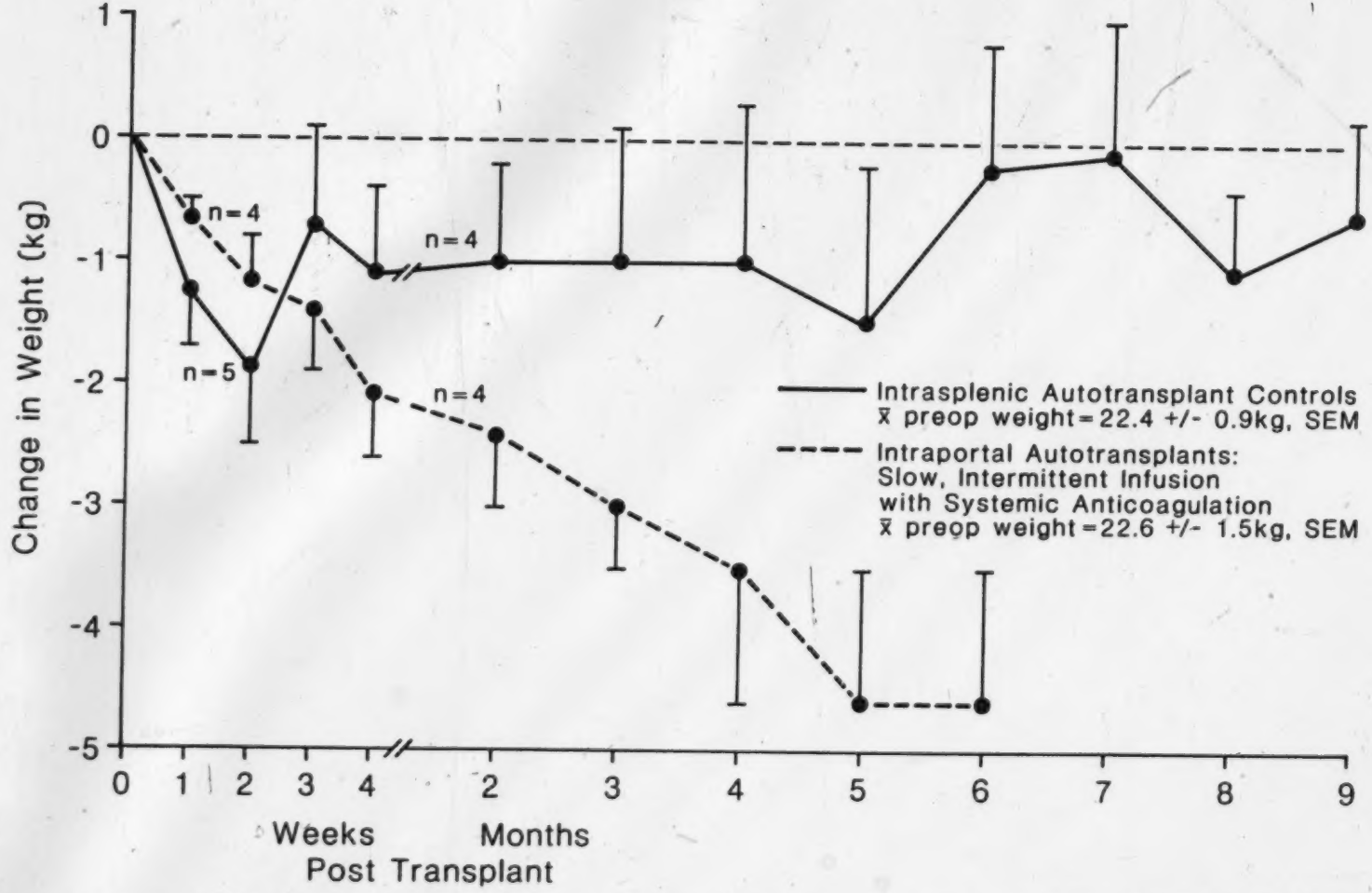

Figure 2 Comparison of postoperative weight change between intrasplenic autotransplant controls and successful intraportal autotransplants.

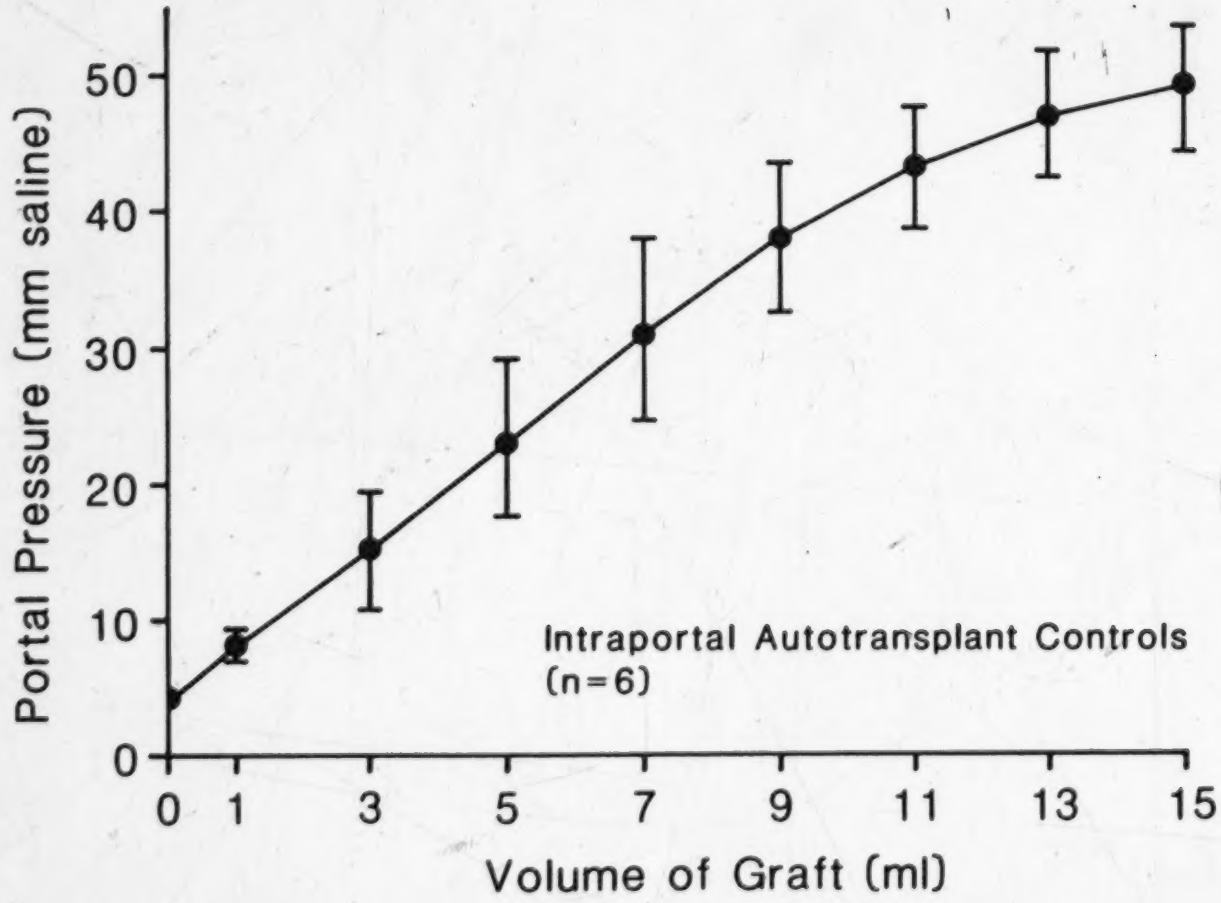

Figure 3 Acute portal pressure changes observed in dogs receiving intraportal infusions of pancreatic fragments.

Plate 1-1: Pancreatic fragments plugging portal vein, less than 24 hours post-transplant. H&E x 400

Plate 1-2: Pancreatic fragments in portal vein with islet (arrow), less than 24 hours post-transplant. A.F. x 400

portal pressures.

Portal pressures returned to baseline in one dog and he survived for 2 months. His mean postoperative glucose was 105 mg % and 1 month K value was 1.7. Hyperglycemia and weight loss developed shortly before a bowel obstruction became evident. At operation there was no evidence of PH; he did not survive resection of a gangrenous intussusception. On microscopic examination of the liver a few portal venules with organized thrombi or pancreatic acini trapped within a fibrous sheath (no islets) were seen (Plates 1-3 and 1-4).

Intraportal Infusion of Enzyme Depleted Graft - As active pancreatic enzymes or peptides may play a role in the development of PH, inert tissue fragments were infused following their fixation in formalin. These 2 dogs also developed acute PH and died. Autopsy and histologic findings were similar to the intraportal autotransplant control group.

Intraportal Autotransplantation: Systemic Anticoagulation or Slow Infusion - A 100 unit/kg bolus of heparin infused prior to the 20 minute embolization or an intermittent graft infusion over 60 minutes in two individual dogs caused the same degree of PH as seen in the intraportal autotransplant control group. When the latter dog was sacrificed 2 hours after transplantation his portal venous tree was completely thrombosed. Within the thrombus clumps of pancreatic fragments were seen (Plate 1-5).

Intraportal Autotransplantation: Slow, Intermittent Infusion with Systemic Anticoagulation - The degree of anticoagulation provided by heparin during the 60 minute infusion period was examined

Plate 1-3: Pancreatic acini within thrombosed portal vein, at 2 months post-transplant. H&E x 630

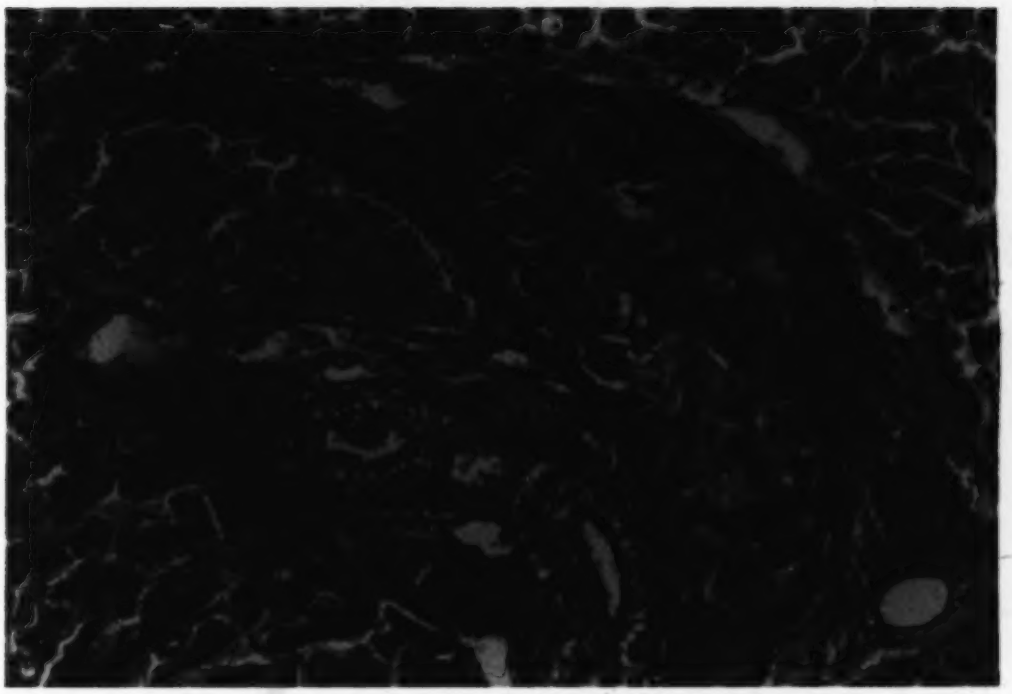

Plate 1-4: Pancreatic fragments in organized portal vein thrombus at 2 months post-transplant. No islets identified. A.F. x 400

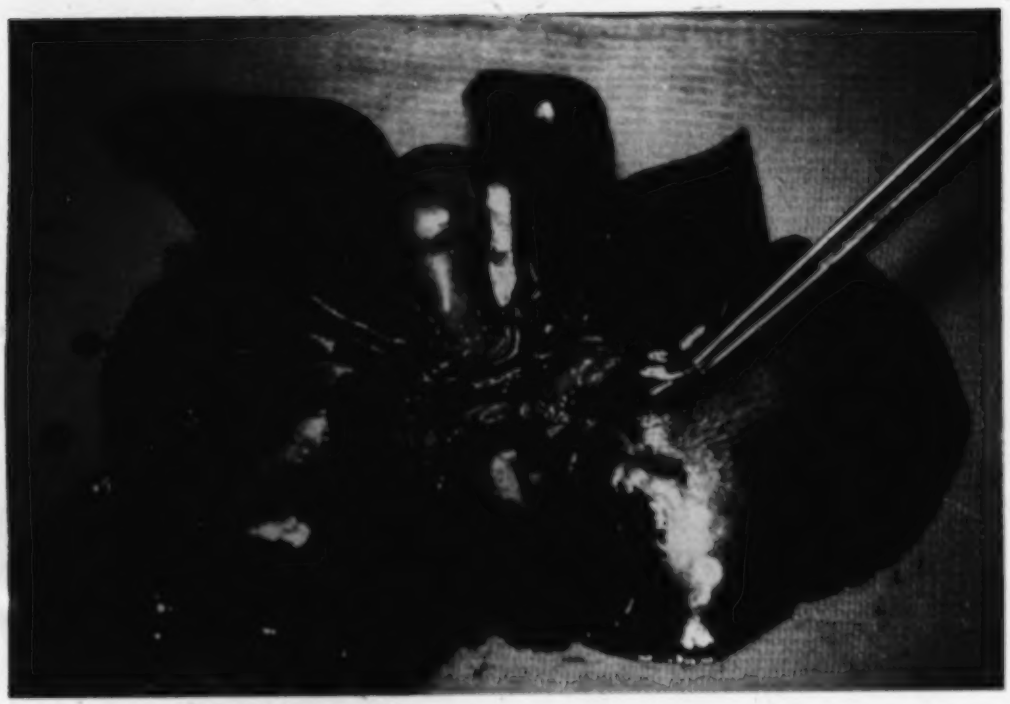

Plate 1-5: Portal vein thrombus in experimental dog undergoing slow, intermittant infusion over 60 min. Sacrificed at 2 hours following transplant.

in 6 healthy dogs of similar age and size and 150 units/kg was chosen (Table 1-3).

Increases in portal pressures were seen, but between the 5 minute infusion intervals pressures would drop and by completion of the transplantation, portal pressures were returning to baseline (Figure 4). The pressures at completion compared to the control infusion pressures were significantly different (29.6 cm H_2O vs 49 cm H_2O).

Initially all dogs were normoglycemic ($\bar{x}$ plasma glucose = 100 $\pm$ 5 mg/dl) but 2 grafts, for unknown reasons, failed early; 3 and 7 days postoperatively. These 2 dogs remained hyperglycemic for 2 months until weight loss necessitated sacrifice. On light microscopy of the liver there was no evidence of the graft or of portal venule thrombosis.

Fasting plasma glucoses were nondiabetic (Figure 1). A steady loss of weight occurred in the 4 long-term survivors (20% at 6 months, Figure 2). K values are shown in Table 1-4.

At 6 months one dog with a satisfactorily functioning graft (K = 1.6, dog #4, Table 1-4) was sacrificed to examine the liver under light microscopy. In spite of searching many sections no islets could be identified. Only a few surviving acinar fragments were seen. The completeness of the previous pancreatectomy was confirmed at autopsy.

One dog remained alive and well at 21 months following transplant (dog #1, Table 1-4). Two others have been sacrificed; one with distemper, (dog #3, Table 1-4) and the other with marked weight loss in spite of normoglycemia (dog #2, Table 1-4).

Table 1-3: Anticoagulation in Response to Increasing Doses of Heparin

Heparin	n	Preinjection	Postinjection	
		PTT in Sec($\bar{x}$)	30 min. PTT in Sec($\bar{x}$)	60 min. PTT in Sec($\bar{x}$)
100 u/kg	3	16	34	25
150 u/kg	1	16	92	49
200 u/kg	2	17	75	58

Table 1-4: K Values Following Successful Intraportal Infusion

	Preoperatively	Postoperatively					
	n=12*	1 mo.	3 mo.	6 mo.	12 mo.	18 mo.	21 mo.
Dog #1		2.0	2.1	3.0	3.2	1.4	1.8
Dog #2		0.8	0.8	0.8	-	-	-
Dog #3		1.5	1.7	0.7	-	-	-
Dog #4		1.4	1.2	1.6	-	-	-
$\bar{x}$	2.8 $\pm$ .16	1.4	1.4	1.5	-	-	-

* Control (n=6) and intrasplenic autotransplant control (n=6) dogs.

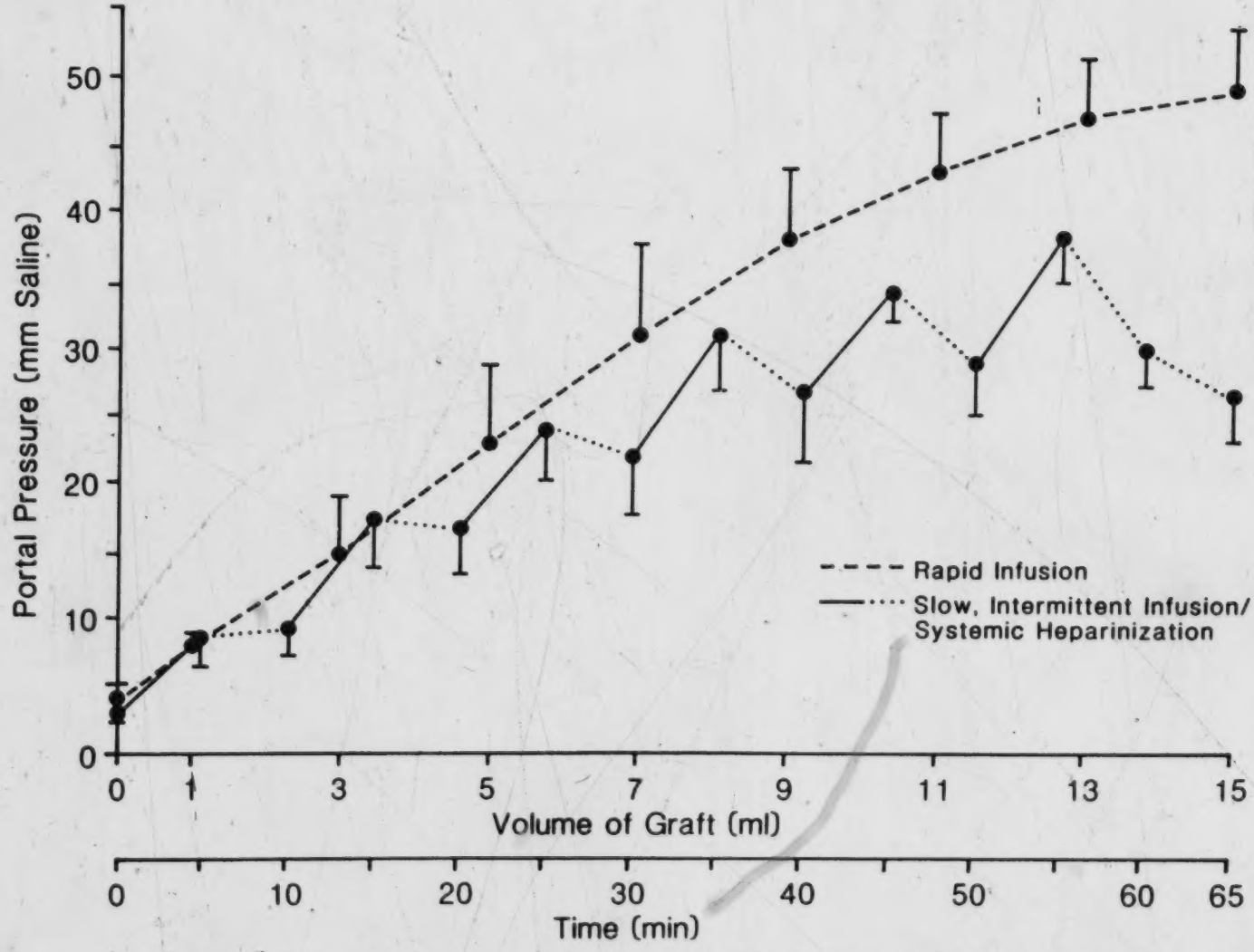

Figure 4 Comparison of acute portal pressure changes seen in dogs receiving intraportal infusions of pancreatic fragments by (i) rapid infusion or (ii) slow, intermittent infusion combined with systemic anticoagulation.

Intraportal Autotransplantation: Tissue Cultured Pancreatic Fragments - The volume of graft recovered following tissue culture ranged from 3.5 - 9 ml. In dogs not receiving heparin 3.5 ml or 5 ml of graft was infused without causing sustained PH. Maximum pressures reached were 13 and 35 cm H_2O respectively but both returned to normal. However, on another occasion 5 ml (as well as 9.5 ml) caused lethal PH (sustained portal pressures >50 cm H_2O).

Slow infusion combined with anticoagulation in 5 dogs receiving 5 to 9 ml of graft allowed only transient portal pressure increases and transplantation survival. The maximum pressure reached was 60 cm H_2O but all elevated pressures returned to baseline.

Histological examination of the liver in those that initially survived (6 days to 8 months) rarely showed any viable exocrine tissue, no islets, and the occasional incompletely or completely thrombosed portal venule.

Development of DIC - There was a slight trend towards the development of DIC in the non-anticoagulated group that was not present in the anticoagulated group. (See Paper #2.)

Discussion

Methods to obtain satisfactory numbers of islets to maintain normoglycemia following islet autotransplantation have been achieved in various animals.[10,20,25-34] Studies in the rat suggest that transplantation in the portal-digestive venous system is particularly important[3] and that the best transplantation site is the liver.[4,5,9,12] No chronic detrimental effects have been seen

following pure-islet intraportal embolization.[35,36]

In the dog the superior transplantation site is not clear.[7,8,9,12] Complicating the intraportal infusion both experimentally (and clinically) is the development of portal hypertension - probably a result of an unpurified mixture of endocrine and exocrine tissue and the resulting larger volume infused.[7,8,9,11-18] Even Banting and Best[37] noted venous thrombosis following intravascular injections of unpurified pancreatic eluate. So not unexpectedly when Warnock et al.[21] tried intraportal infusion of pancreatic fragments all dogs developed portal hypertension and none survived to compare with the very successful intrasplenic autotransplant group. A method has now been developed that allows survival after intraportal infusion of pancreatic fragments. Comparisons in function and survival between intraportal and intrasplenic transplantation can be made.

This gland preparation recovered satisfactory numbers of islet-bearing pancreatic fragments so that 5 of 6 dogs receiving intrasplenic transplants remained normoglycemic and essentially maintained their weight. K values over a 6 month period were non-diabetic and showed no evidence of deterioration. PH was not a complication of this infusion. du Toit[38] has noted PH from accidental intraportal embolization of pancreatic fragments initially transplanted to the spleen.

Intraportal embolization of pancreatic fragments over 20 minutes caused marked, sustained increases in portal pressures and proved lethal for 5 of 6 dogs. On histological exam almost all portal venules were occluded with graft, or graft and surrounding thrombus.

Changes of hepatic ischaemia were marked. For unknown reasons one dog that was infused with a similar volume of graft (17 ml) reached comparable portal pressure elevations (49 cm H_2O) but this pressure had almost returned to baseline by the time of abdominal closure. He remained normoglycemic for 2 months until his graft failed in a short period of time. This may have been related to the stress of a chronic but fatal bowel obstruction. When hepatic tissue was examined by light microscopy several portal venules were partially filled with organized thrombus containing degenerating exocrine tissue. In none of the multiple sections were islets visible.

Infusion of the wash solution from pancreatic fragment preparation, known to be rich in thromboplastins,[11] did not cause an increase in portal pressures. However, tissue that was devoid of active enzymes or peptides following formalin fixation but of similar volume caused a comparable degree of PH as did the infusion of fresh graft. Portal pressure elevations were sustained and greater than 50 cm H_2O. It seems possible that 2 components of Virchow's triad (of venous thrombosis) are present here: (i) stasis as a result of physical obstruction caused by the large volume of embolized pancreatic fragments; (ii) a localized hypercoagulable state knowing the pancreatic fragments and damaged cells could act as potent thromboplastins. There may also be a change in the portal venule walls if active exocrine enzymes were being released by the fragments only to injure the vessel wall. Combining these factors could cause the massive intraportal thrombosis as seen here (Plate 1-5). Because as little as 5 ml of tissue caused the same degree of portal

hypertension and was lethal it seems the induced hypercoagulable state leading to thrombosis may be very potent.

The use of slow, intermittent infusion made little difference to the development of PH, but failure of systemic anticoagulation with a 20 minute infusion was unexpected. However, the combination of slow, intermittent infusion with systemic anticoagulation allowed intraportal graft infusion without causing sustained PH (Figure 4). Six of six dogs survived and were immediately normoglycemic. There is no obvious explanation for the early failure of 2 grafts even though these dogs went on to live for 2 months suggesting minimal graft function. Autopsy specimens of liver examined miscroscopically showed the portal venules to be devoid of either endocrine or exocrine tissue.

Although fasting plasma glucoses were non-diabetic and similar to the intrasplenic group, a concerning loss of weight (20% by 6 months) occurred in the 4 surviving dogs. One dog with a K value of 1.8 at 21 months has maintained his weight. One dog remained normoglycemic but never had a K value above 1 and he showed progressive weight loss. In another dog, weight loss coincided with a diagnosis of distemper. His 1 and 3 month K values were 1.5 and 1.7 respectively. This deteriorated to 0.7 at 6 months when distemper was evident. The fourth dog with consistently satisfactory K values was sacrificed at 6 months to examine the liver histologically (Dog #4, Table 1-4). His weight loss was 6.3 kg (24%). In spite of scanning numerous microscopic sections no intra- or extraportal[4,39] islets were identified. Only remnants of exocrine tissue within portal venules were visible. The hepatic parenchyma was normal suggesting

there was no chronic complication from the graft infusion.

The transplantation of tissue cultured fragments suggested that when a volume 3.5 ml was to be infused this should be accompanied by anticoagulation and slow infusion. Microscopically it was unusual to find evidence of the intraportal graft. Most dogs were sacrificed just a few weeks (2-8) following transplantation because of weight loss and hyperglycemia.

This study confirmed the ability to transplant pancreatic fragments into the liver without developing sustained PH. Chronic complications are not anticipated as other studies have only shown minor, transient liver enzyme changes, normal hepatic architecture[8,14,34,35,36] and normal portal pressures on follow up.[9] However, like the experience of Kretschmer,[9] this graft preparation may not function as well on long-term follow up when transplanted into the liver instead of the spleen.

An ominous finding is the degree of organized thrombus surrounding intraportal fragments, and how, just a few weeks after transplantation pancreatic fragments are often difficult to identify. Venous thrombi with surrounding organization enveloping the islets and acini has been noted by others.[16,19] Islet tissue is initially felt to be kept alive by nutrient diffusion until neovascularization occurs.[40,41] But if the fragments, and/or exocrine secretions stimulate thrombosis, organization of the clot could lead to ischemia of the islets and insulin production might fail. In a similar manner it has been proposed that failure of islet grafts obtained from a gland diseased with chronic pancreatitis is partly due to the fibrosis

and scaring immediately surrounding the islets. This subsequently interferes with their neovascularization following transplantation.[15,42]

The future for intrahepatic transplantation probably lies in obtaining a more purified islet preparation. Evidence in rats[4] and limited experience in dogs[10] using pure islets suggests the smaller volume of tissue and smaller fragments are tolerated easily and succeed in the long term.

Conclusions

1. Pancreatic fragments transplanted intraportally will cause portal hypertension.
2. With systemic anticoagulation and slow intermittent infusion pancreatic fragments can be transplanted intraportally with long term survivors.
3. Autotransplanted pancreatic fragments, either via an intrasplenic or intraportal route, will maintain fasting normoglycemia and tolerance to intravenous glucose. However, weight loss is more of a problem with intraportal transplantation.

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INTRAPORTAL AUTOTRANSPLANTATION OF PANCREATIC FRAGMENTS: IS DIC ALWAYS A PROBLEM?

Introduction

The transplantation of islet tissue to prevent the long-term complications of diabetes mellitus is an attractive concept.[1] It has been more than a decade since Ballinger and Lacy[2] successfully isolated sufficient numbers of pure islets to reverse hyperglycemia following transplantation in the diabetic rodent. Shortly afterwards transplantation within the hepatoportal circulation was shown to be advantageous[3] with the liver being the best graft-recipient site.[4]

A major advance was a simplified isolation procedure that allowed one pancreas to act as a single islet donor. This technique sacrificed islet purity for a mixture of islets and exocrine tissue that was both tolerated and successful when transplanted to the spleen.[5] But intraportal infusion of mixed endocrine/exocrine fragments in either experimental or clinical trials has led to the development of portal hypertension (PH).[6-16] This complication has not been seen with relatively pure islet grafts.[17,18] Another major complication is the development of disseminated intravascular coagulation (DIC).[10,13,14]

DIC develops as an excessive reaction in the normal equilibrium between coagulation and fibrinolysis. When these two mechanisms are over-stimulated there is a consumption of coagulation factors and platelets, microvascular fibrin deposition, damage to red cells, diffuse organ injury and excessive bleeding. It can range from a laboratory diagnosis to severe clinical manifestations and death.[19]

Using a standard preparation of mixed endocrine and exocrine fragements[5] Mehigan et al.[10] noted severe PH and DIC following intraportal transplantation in a patient and subsequently in a series of experiments in dogs. There were marked changes in the platelet count and fibrinogen level, a prolonged PTT, development of FDP's, and significant hemorrhage. This was subsequently prevented by the addition of heparin and aprotinin to the graft prior to infusion. It was demonstrated that tissue thromboplastins released from the gland during preparation were probably responsible.

Others have not noted DIC to be such a problem when using a similar islet isolation technique.[6,8,9,11,12,15,16] We reported our experience using a gland preparation that was similar to that of Warnock et al.[20] which also documented PH as a major problem following intraportal infusion of mixed pancreatic fragments.[21] The development of DIC was not evident.

Materials and Methods

Outbred, adult mongrel dogs, 1-2 years of age weighing 15-25 kg were studied. Criteria of the Canadian Council on Animal Care were followed.

All dogs underwent a total pancreatectomy and autotransplantation to the spleen (n=2) or liver (n=24) following 2-2 1/2 hours of pancreatic fragment preparation.[20] Eight of the dogs receiving an intraportal infusion had grafts remain in tissue culture for 24-48 hours to reduce both the exocrine component and total graft volume.

The preparation of mixed endocrine and exocrine pancreatic

fragments began with dissecting out the gland while maintaining its major vascular supply. The major and minor pancreatic ducts were cannulated in situ and following excision the gland was weighed. It was then distended and perfused with chilled (4°C) Hanks balanced salt solution (HBSS-Gibco, Grand Island, N.Y.). A ≃ 30 minute perfusion with 0.4% collagenase followed and when the gland appeared mucoid it was chopped for 90 sec. In supplemented HBSS (penicillin G 100,000 units/ℓ, streptomycin 100,000 ug/ℓ, aprotinin 200,000 KIU/ℓ) it was vigorously shaken in an iced water bath and passed through a 400u screen x3. After washing x3 it was resuspended in the supplemented HBSS for immediate infusion or transferred to tissue culture flasks.

In 8 dogs gland processing was followed by placing the entire graft in tissue culture. Sterile technique was used. RPMI 1640 (Gibco, Grand Island, N.Y.) was chosen as tissue culture media; 100 ml media per ml of tissue. This was supplemented with heat inactivated fetal calf serum 10% v/v (Gibco). Penicillin, Streptomycin and aprotinin were added in the same concentrations as they were to the HBSS during gland preparation. HEPES (Gibco) was added to a concentration of 25 mM/ℓ as a pH buffer. Tissue was placed either in multiple static tissue culture flasks or was kept suspended in continuous motion by a magnetic spinbar in a 2ℓ Fernbach flask driven by an external spinner. The cultures were placed in a 5% CO_2/room air humidified environment. Tissue was kept in culture for 24-48 hrs. (with a media change at 24 hrs.) and then pelleted and resuspended in supplemented HBSS for transplantation. Media pH and bacterial cultures were routinely checked.

Portal pressures and hematological tests to evaluate DIC were examined in all groups. Two dogs received tissue embolized over 10 minutes via small hilar veins to the spleen. Six control dogs underwent continuous intraportal graft infusion over 20 minutes. Two dogs received an intraportal infusion of graft fixed in formalin to remove any active enzyme or peptide component. It was washed several times prior to infusion. In trying to find a method of infusion that did not cause death secondary to portal hypertension (and sometimes portal vein thrombosis) two other techniques were tried: (i) systemic anticoagulation with 100 units/kg heparin using a 20 min. infusion, (ii) a slow, intermittent 60 min. infusion. Combination of these two techniques alleviated the lethal sustained PH and a series of 6 dogs anticoagulated with 100-150 units/kg of heparin received grafts infused over a 60 minute period. Prior to developing this technique 3 dogs received cultured fragments without anticoagulation and then a further 5 dogs followed the successful heparinization/infusion protocol. In the anticoagulated groups 30 units of heparin/ml of recovered tissue was added to the suspension of mixed endocrine/exocrine fragments.

Portal pressures were continuously measured by a simple water manometer zeroed with the dogs' midaxillary line. The manometer was connected by IV tubing to the same 16 gauge silastic catheter used for the intraportal graft infusion.

The laboratory diagnosis of DIC was based on monitoring changes in the PT, PTT, platelet count, fibrinogen level and the detection of fibrin degradation products (FDP's).[22] Samples were drawn after

induction of anesthesia, immediately before and at the completion of graft infusion (about 6 hours after anesthetic induction) and finally after closing the incision (1/2 hour after completing the infusion). Minimal changes were noted from the time of anesthetic induction to just prior to graft infusion so only the pre-injection and 30 minute post-transplant values are reported. There was little difference in values at the completion of infusion and 30 minutes later. Peripheral smears were examined for schistocytes. Note was made of petechia formation, abnormal clotting or hemorrhage at the time of operation or autopsy. Renal glomeruli were examined microscopically for fibrin thrombi.[23,24]

Normal values for the hematologic tests were established in healthy mongrel dogs of similar age and weight (n=10 to 16).

PT's and PTT's were done manually on a Fibrometer (Fibrotek Co.) using Simplastin and Automated APTT reagents (General Diagnostics, Morris Plains, N.J.). Platelet counts were done on a TOA Automatic Platelet Counter-Model PL-100 (TOA Medical Electronics Co., Kobe, Japan) with adjustment for the size of dog platelets. Fibrinogen was determined by the method of Ratnoff and Menzie[25] using a Turner Model 350 Spectrophotometer (Turner, Palo Alto, Ca.). FDP's were measured using the Thrombo-Wellco test (Wellcome Diagnostics, Dartford, Eng.).

All dogs that did not survive underwent autopsy. Tissue was fixed in 10% formalin, embedded, processed and stained with H&E in the standard fashion.

Statistical Analysis

All values are expressed as means with the standard error of the mean (SEM). Comparison of group results was done with paired or unpaired Student's t-tests. Differences are stated as significant when $p < 0.05$ unless indicated otherwise.

Results

The volume of tissue obtained from graft preparation that was subsequently transplanted is outlined in Table 2-1. The volume of tissue recovered after tissue culture for the non-heparinized and heparinized groups is also included. There were no significant differences between the non-tissue culture groups in the volume of tissue recovered prior to immediate infusion. Also, between the tissue culture groups the volume of tissue infused was similar.

Included in Table 2-2 are the normal ranges for the coagulation parameters from this dog population and the normals as reported in the literature.[22]

No complications had been encountered in previous intrasplenic infusions so only 2 dogs had hematologic studies performed. Differences were minimal between pre- and postinfusion values.

Infusion of pancreatic fragments into the portal vein of the control dogs was lethal for 5 of 6 dogs. PH was severe and sustained[21] (49+ cm H_2O vs 4 cm H_2O baseline). Petechiae were seen in the mesentery but bleeding from the wound edge, venipuncture sites, suture holes or airway (endotracheal tube) was not noted. At autopsy only a small amount of slightly sanguinous intraperitoneal

Table 2-1: Volume and Percent Recovery of Tissue Fragments

Group	n	$\bar{x}$ vol. (mls)	% Recovery
Intrasplenic Infusion	2	16 ± 1	29 ± 2
Intraportal Infusion			
Controls	6	16 ± 1	28 ± 2
Modified Technique	4	18 ± .5	30 ± .8
Tissue Culture - No heparin	3	5 ± .5	-
Anticoagulation/60 min. infusion	6	16 ± 1	32 ± 4
Tissue Culture - Heparin/ slow infusion	5	8 ± .8	-

Table 2-2: Normal Values for Coagulation Tests

Group	PT (sec) n=16	PTT (sec) n=16	Platelets x 10^3 n=10	Fibrinogen (mg/dl) n=14
Mongrel dogs	8.7 ± .2	15.8 ± .4	239 ± 12	260 ± 23
Quoted Literature	6.4 - 7.4	9.5 - 10.5	200 - 500	200 - 400

fluid was found. On light microscopy there was evidence of severe hepatic ischaemia (Plate 2-1) but no fibrin thrombi were found in renal glomberuli (Plate 2-2). Changes in coagulation tests were evident (Table 2-3). The slight prolongations of the PT and PTT were not significant nor was the drop in figrinogen. The drop in platelet count at postinfusion was significant (203,000 to 140,000) but by 30 min. postinfusion the platelet count had risen and the difference was no longer significant (203,000 to 161,000, $p = .10$). Only one dog had FDP's between 10 and 40 ug/ml; all others were negative. Study of peripheral smears showed occasional schistocytes.

The individual results using graft fixed in formalin, slow infusion or systemic anticoagulation with a 20 minute infusion are included in Table 2-4. The differences between pre- and postinfusion values were similar to the intraportal control group except the drop in platelet count was a little greater (average drop of 81,000 compared to 63,000). The degree of PH, appearance of petechiae and other findings were similar to the intraportal control group. The latter 2 dogs (slow infusion only/systemic anticoagulation only) were sacrified 2 hours following transplantation when it became apparent they would not survive. The portal tree was completely thrombosed in both dogs.

Infusion of smaller volumes of tissue cultured fragments ($\bar{x}$ vol. = $5 \pm .5$ ml) allowed 2 of 3 dogs to survive. One dog died of acute PH shortly following completion of the infusion. Clinically there was no suggestion of DIC. Coagulation results are given in Table 2-5. The changes in PT, PTT, platelet count and fibrinogen level were not

Plate 2-1: Severe hepatic ischaemia seen following intraportal embolization of graft leading to sustained PH. H&E x 400

Plate 2-2: Renal glomeruli without fibrin thrombi. Autopsy sections following death from severe, sustained PH. H&E x 200

Table 2-3: Coagulation Studies of Intraportal Infusion-Control Group.

Group	PT (sec) n=6	PTT (sec) n=6	Platelet Count x 10^3 n=4**	Fibrinogen (mg/dl) n=4**	FDP's (ug/ml) n=6$
Pre-Infusion	10.1 ± 1.7	16.8 ± 1.8	203 ± 45	252 ± 85	neg
Postinfusion	-	-	140 ± 37	-	-
30 min Post-infusion	12.7 ± 5.4	17.3 ± 1.5	161 ± 38	159 ± 82	neg
Difference	+2.6[t]	+0.5*	-63/-42	-93	-

Value reported as mean ± S.D.
t one value prolonged
* two values prolonged
** lab error negated 2 values
$ one value 10 - 40

Table 2-4: Coagulation Studies of Intraportal Infusion - Modified Techniques.

Group	n	PT (sec) Pre	PT (sec) Post	PT (sec) Diff	PTT (sec) Pre	PTT (sec) Post	PTT (sec) Diff	Platelets x 10^3 Pre	Platelets x 10^3 Post	Platelets x 10^3 Diff	Fibrinogen (mg/dl) Pre	Fibrinogen (mg/dl) Post	Fibrinogen (mg/dl) Diff
Enzyme Depleted	1[+]	8.9	9.2	+0.3	22	24	+2	341	255	-86	421	206	-215
Slow Infusion	1	10.7	11.4	+0.7	16	16	0	232	144	-88	218	157	-61
Anti-coagulation	1	11.2	12.6	+1.4	16	25*	+9*	256	186	-70	156	88	-68

+ Samples on one dog not shown.
* heparinized

Table 2-5: Coagulation Studies of Intraportal Infusion - Tissue Cultured Pancreatic Fragments

Group	PT (sec) n=3	PTT (sec) n=3	Platelets x 10^3 n=3	Fibrinogen (mg/dl) n=3
Pre-Infusion	9.5 ± 0.6	20 ± 1.0	276 ± 55	342 ± 104
Postinfusion	9.9 ± 0.4	21 ± 1.2	220 ± 27	294 ± 167
Difference	+ 0.4	+1	-56	-48

significant.

Combining systemic anticoagulation with an intermittent infusion over 60 min. avoided the development of marked or sustained elevations in portal pressures (29.6 vs 49 cm H_2O).[21] Clinically DIC was not a problem. Coagulation results are presented in Table 2-6. There were no significant changes in the PT, platelet count or figrinogen level.

The differences in the coagulation changes pre- and 30 min. postinfusion between the intraportal control group and the heparinization/intermittent infusion group were examined. Changes in the PT between the 2 groups were similar. There was a significant difference between the 2 groups in the change in the platelet counts. There was a significantly smaller change in the heparinization/ intermittent infusion group (a decrease in platelet count of 14,000 vs 42,000). The difference between the 2 groups in the change in fibrinogen was almost significant (p=.10); again a smaller change was seen in the heparinization/intermittent infusion group (a decrease in fibrinogen of 2 mg/dl vs 93 mg/dl).

The successful infusion technique was then applied to additional transplantations using tissue cultured fragments. These dogs had transient increases in portal pressures, no clinical evidence of a coagulopathy and all survived. Differences in coagulation tests pre- and postinfusion (Table 2-7) are not significant.

Table 2-6: Coagulation Studies of Intraportal Infusion - 60 min. Infusion with Anticoagulation

Group	PT (sec) n=6	PTT (sec) n=6	Platelet Count x 10^3 n=6	Fibrinogen (mg/dl) n=6
Pre-Infusion	9.9 ± 0.9	15.5 ± 1.5	187 ± 27	162 ± 39
Postinfusion	10.3 ± 1.0	43 ± 22*	173 ± 34	160 ± 27
Difference	+0.4	+27*	-14	-2

Values reported as mean ± S.D. * Heparinized

Table 2-7: Coagulation Studies of Intraportal Infusion - Tissue Cultured Pancreatic Fragments: 60 min Infusion with Anticoagulation

Group	PT (sec) n=5	PTT (sec) n=5	Platelets x 10^3 n=5	Fibrinogen (mg/dl) n=5
Pre-Infusion	9.8 ± 0.7	17.2 ± 2	233 ± 54	340 ± 154
Postinfusion	9.8 ± 0.8	23.6 ± 7*	226 ± 57	275 ± 107
Difference	0	+6.4*	-7	-65

Values reported as mean ± S.D. * Heparinized

Discussion

Transplantation of islet tissue in diabetics receiving a kidney transplant or in patients undergoing a 95% pancreatectomy for chronic pancreatitis is already a reality.[26] Unfortunately, transplantation of islets into the liver, guided by favourable results in rodents,[4] has led to serious complications that sometimes have been fatal.[14,16] The critical difference is pure islets were used in rats whereas in clinical trials an unpurified mixture of islet and exocrine tissue is all that is currently available. The lack of purification results in a much larger volume of tissue to be infused. As a result of transplanting a mixture of endocrine and exocrine tissue into the liver both PH and DIC have occurred as complications.

PH has occurred in almost all animal experiments and clinical trials of intraportal infusion using pancreatic tissue fragments,[6-16] but DIC is fortunately less common. DIC is not new to the field of transplantation.[17] DIC is basically an excess of the coagulation and fibrinolytic mechanisms, both of which modulate the other.[24] It usually follows a stimulus to the extrinsic coagulation pathway and can be acute or chronic. The result is a consumption of clotting proteins, thrombocytopenia, FDP production and microvascular fibrin deposition which may damage red blood cells or cause diffuse end organ damage.

DIC might be anticipated as a complication, knowing the relatively large volume of tissue being infused, and in particular the location - the portal venous tree. The liver plays a major role in

maintaining a normal liquid state of blood and hemostatic capability. It is responsible for synthesizing several of the clotting factors and fibrinogen. It also clears from the blood tissue activators of clotting and fibrinolytic enyzmes. In the presence of hepatic ischaemia, clotting protein levels may drop and those products that lyse blood may circulate in excess,[28] leading to a hypocoaguable state which is the opposite of the normal postoperative hypercoagulable milieu.[29]

A comprehensive study of DIC following pancreatic fragment transplantation was performed by Mehigan et al.[10] after noting this complication in a patient. Observations from a canine model led to the suggestion that tissue thromboplastins were released during the gland preparation and upon infusion they were a trigger for the development of DIC. If heparin and Trasylol (aprotinin - a nonspecific proteinase inhibitor) were added to the graft suspension prior to infusion, DIC was no longer evident. This was followed by successful clinical application. Most other groups have now gone on to use heparin, but systemically[8,9,11, 15] rather than in the graft.[13] In some circumstances heparinization has not been found necessary.[12] The extension of information gained from intraportal infusion of pancreatic fragments in dogs may not be entirely applicable to humans because of the dogs' extremely sensitive splanchnic vascular bed and the presence of hepatic venous sphincters.[28]

In this study the lack of consistent development of significant changes in the PT, PTT, platelet count, fibrinogen level and minimal

to absent generation of FDP's suggests that DIC is not occurring. There are no absolute criteria to diagnose the presence or absence of DIC. Reliance is placed on a combination of appropriate changes under the correct circumstances.[23,30] (Commonly there is an elevation of one or both of the PT, PTT; a drop in the platelet count and fibrinogen level and clinical occurrence of abnormal clotting or diffuse hemorrhage). However most would agree that FDP's should be present and usually greater than 40 ug/ml.[22, 23] The presence of petechiae, hemorrhage or organ dysfunction is not always present although more common in the acute form of DIC. In the control group the relatively minor changes in the PT or PTT combined with the virtual absence of FDP's and lack of clinical problems or fibrin thrombi in renal glomeruli argues against DIC being a complication of this pancreatic fragment transplantation model. The decline in fibrinogen (252 - 159 mg/dl), although not significant, is notable considering in the usual postoperative state it should rise.[29] Interestingly the platelet count was consistently lowest at the end of the infusion but quickly began to rise just 30 minutes later.

Another point negating DIC as an explanation for the coagulation changes was the presence of portal vein thrombosis, as seen on two occasions. If autopsies had been done immediately after death this observation may have been made more frequently. Major vessel thrombosis is distinctly uncommon in DIC.[19,31]

When systemic anticoagulation was combined with a slow, intermittent infusion the significant difference was an absence of sustained PH and subsequently all dogs survived. This difference was

reflected in the coagulation tests - the changes in the PT, platelet count and fibrinogen level were less (Table 2-6) and none reached statistical significance. Petechiae were not apparent in the mesentery but their absence is more likely a reflection of the decrease in portal pressure. The one dog that survived in the intraportal infusion control group also had only a transient increase in portal pressures (and coagulation changes that were minimal).

The complication of this intraportal infusion of pancreatic fragments is mainly the development of PH.[21] Any coagulation changes are probably more a result of local portal vein thrombosis and the accompanying ischaemia to the liver. The embolization of such a large volume of tissue fragments leads to portal venule obstruction, stasis, and when combined with the tissue thromoboplastins in the suspension leads to intravascular clotting. It seems enzymes associated with the exocrine component of the graft could contribute to clotting by causing endothelial injury or direct conversion of prothrombin to thrombin,[32] but the same lethal results from infusing formalin treated tissue suggests in the acute phase enzymes are not an essential cause of clotting. Also, the infusion of tissue decreased in enzyme content following tissue culture (our observation) was still lethal unless the volume of tissue was greatly reduced (3.5 - 5.0 ml). Matas et al.[7] had a similar experience with tissue cultured fragments in that systemic anticoagulation was still required and not all of the graft could be infused intraportally.

It appears as if any coagulation changes noted here are not representative of DIC. A plausible explanation is the changes are a

consequence of portal vein thrombosis and hepatic ischaemia occurring secondarily to acute, sustained PH. All of this could be avoided by infusing either a small volume of tissue or anticoagulating the dog at the time of a slow infusion.

Conclusions

1. Islet containing pancreatic fragments, when infused into the liver via the portal vein, cause minor coagulation changes.
2. The changes appear to be related to the occurrence of acute, sutained PH and in its absence are less apparent.
3. Heparin can be used as a systemic anticoagulant to allow safe infusion of the pancreatic fragments and neither sustained PH or coagulation changes suggestive of a coagulopathy will be seen.

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DISCUSSION AND CONCLUSIONS

Although transplantation remains the definitive technique to replace the function of a failed organ only kidney or corneal allografts (and blood transfusions) are routine. The techniques of transplanting the heart, heart/lungs, liver or bone marrow have been established in several research centers. However, the transplantation of pancreatic endocrine tissue to prevent the long term complications of diabetes mellitus is still very much an experimental method.[1]

Currently, the pancreas is usually transplanted as a segmental gland,[2] but the accompanying exocrine tissue is undesirable, and efforts to isolate pure islets for transplantation continue.[3]

Ballinger and Lacy[4] showed isolated islets could be retrieved in sufficient numbers, transplanted, and return the diabetic rat to a normoglycemic state. The importance of the liver as the recipient site for transplanting islets was shown by Kemp,[5] Feldman[6] and Brown[7]. As human trials began, it was realized an intermediate sized animal to advance techniques was required and usually a dog model was chosen. In an attempt to isolate sufficient islets from a single gland, it was realized that complete separation of endocrine and exocrine tissue was not necessary.[8] This allowed satisfactory embolization of tissue to the spleen but when infused into the liver portal hypertension (PH) and sometimes systemic hypotension or disseminated intravascular coagulation (DIC) were seen.[9-12]

PH, an increase in pressure within the venous tree draining the digestive tract to the liver, can be anticipated knowing that frequently 10-20 ml of tissue fragments were being infused into the

dogs' portal vein. This large volume combined with the dogs' sensitive splanchnic vascular bed and hepatic venous sphincters[13] which could respond to vasoactive amines possibly released from the gland preparation could easily raise venous pressure.

DIC is an exaggerated response to a coagulation stimulus. There is an overwhelming reaction leading to the consumption of clotting factors and platelets leading to microvascular fibrin deposition. Products of the coagulation cascade stimulate the fibrinolytic mechanism which normally helps prevent widespread, unchecked clotting. The breakdown products of fibrinolysis - fibrin degradation products or FDP's - in excess further interfere with the formation of clot. This results in a paradox of microvascular thrombosis interfering with organ function and a hypocoagulable state leading to diffuse hemorrhage.[14]

It was determined that both of these problems could be avoided by the administration of heparin as an anticoagulant,[11] either in the graft suspension or given systemically. These technqiues have been adopted by most researchers[15-18] and have met with some clinical success, especially in the autotransplant patient following a 95% pancreatectomy for the pain of chronic pancreatitis.[17]

In the dog model most groups have followed a gland preparation technique as outlined by Mirkovitch & Campiche[8] - essentially ductal distention, mincing and then collagenase digestion.[9,12,15,19] There is little long-term follow up data to compare with the experience of a model developed by Warnock et al.[20] He had 7 of 9 long-term survivors at 5 months with K values (% decline in glucose/min

following a glucose challenge) of 1.4 to 1.5. Several dogs have remained normoglycemic at 2-3 years and appear healthy.[21]

Warnock's experience was confirmed here, as it has been by others.[22] Five of six dogs receiving intrasplenic autotransplants were immediately normoglycemic and remained well[+] with K values of 1.4 at 6 months. But the development of PH upon intraportal graft infusion was similar to the experience of others.[9,10,11,15,16] All dogs developed acute PH which was sustained in 5 and they all died. One dog with transient PH went on to live for 2 months, for the most part normoglycemic prior to developing a lethal bowel obstruction. In the dogs that did not survive changes in the coagulation tests occurred but were not of significance. Bleeding was not a problem and at autopsy fibrin thrombi were not seen within renal glomeruli. Acute hepatic ischaemia was evident. In the lone survivor coagulation changes were minimal.

The same results occurred following infusion of enzyme depleted/formalinized graft suggesting that the physical presence of the tissue fragments and not so much its active enzyme or peptide components was responsible for the development of PH. When small volumes of tissue were infused following tissue culture (which would decrease the enzyme content 3-4 fold)[23] again fatal PH would develop if 5.0 ml of tissue or more was infused.

If the infusion was prolonged over 1 hour or the dog was anticoagulated during a 10 to 20 minute infusion the results were

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[+] One dog died of a bowel obstruction.

unchanged and in individual experiments neither dog survived. But the combination of anticoagulation with slow intermittent infusion caused only transient PH in 6 of 6 dogs. Coagulation changes were negligible. All dogs survived and were immediately normoglycemic. For unknown reasons, the graft function failed quickly in 2 recipients and they survived for only 60 days. The remaining 4 dogs as a group did well but weight loss was a problem, losing an average of 4.6 kg or 20% body weight. Individually one dog has done very well with a K value at 21 months of 1.8 and no weight loss. Another dog (always normoglycemic) had diabetic K values and was sacrificed at a cummulative steady weight loss of 25%. Another dog developed distemper and subsequently hyperglycemia. The last dog was sacrificed at 6 months (K value = 1.6) to examine the liver histologically. In spite of examining multiple sections of the hepatic parenchyma no evidence of islets, either within portal venules or the adjacent hepatic lobules could be found. Only a few fragments of exocrine tissue were present.

The reason why intraportal pancreatic fragment transplants do not function any better or possibly not as well as intrasplenic transplants may lie in the interpretation of miscroscopic findings in the liver. It was not uncommon to find partially thrombosed portal venules, often with a few elements of degenerating exocrine tissue in the center of the thrombus. Islets were rarely seen. The longer the time from the transplant, the less tissue or thrombus would be seen within venules. This suggests that the embolized tissue quickly becomes enveloped in thrombus which over a period of time becomes

organized fibrous tissue (almost devoid of vascularity). Islets, when transplanted as tissue fragments, initially depend on nutrient diffusion for their survival and by 10-14 days neovascularization has occurred.[24,25] If thrombus and its subsequent fibrous organization interfers with the viability of the islet, then over a period of time the islets would become ischaemic and their function would fail. Hyperglycemia, weight loss and the complex of diabetes mellitus would soon develop. Heavy thrombus formation and fibrous scaring could also interfere with islet migration beyond the portal venule to the hepatic parenchyma, a process recognized in the rat transplanation model.[25,26]

The exocrine tissue certainly appears to be a liability. Without it PH or DIC is not a problem when employing an intraportal infusion technique.[4,27,28] With exocrine tissue as part of the graft there appears to be a stimulus to thrombus formation which could interfere with both neovascularization or islet migration. Also, trypsin (found in exocrine tissue) is known to convert prothrombin to thrombin,[29] and this could provide an additional continuous stimulus to the clotting mechanism and subsequent thrombus formation.

If the liver is to be shown to be the optimum site of islet transplantation, it appears that further efforts must be made in developing islet-isolation techniques that provide a more pure islet graft. Only then can the results obtained in the rat islet transplantation model be anticipated in large mammals including human clinical trials.

In conclusion:

1. The Warnock et al. method of pancreatic fragment preparation for intrasplenic infusion causes portal hypertension and mild changes in coagulation parameters when infused into the liver via the portal vein.
2. With systemic anticoagulation and slow, intermittent infusion pancreatic fragments can be transplanted intraportally and result in long term survivors.
3. The autotransplantation of mixed endocrine and exocrine either to the spleen or liver will normalize plasma glucose but neither site is clearly superior to the other.

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